

Patent Claims

1. A method for the analysis of breast cell proliferative disorders by determination of the methylation state of one or more sequences from the group of Seq. ID No.1 through Seq. ID No. 73 and Seq. ID No. 366.
2. A method according to Claim 1, said method comprising contacting a nucleic acid consisting essentially of one or more sequences from the group of Seq. ID No. 1 through Seq. ID No. 73 and Seq. ID No. 366 in a biological sample obtained from a subject with at least one reagent or a series of reagents, wherein said reagent or series of reagents, distinguishes between methylated and non methylated CpG dinucleotides within the target nucleic acid(s) from the group of Seq. ID No.1 through Seq. ID No. 73 and Seq. ID No. 366.
3. A nucleic acid molecule consisting essentially of a sequence at least 18 bases in length according to one of the sequences taken from the group comprising Seq. ID No. 74 through Seq. ID No. 365 and Seq. ID No. 367 through Seq. ID No. 370 and sequences complementary thereto.
4. An oligomer, in particular an oligonucleotide or peptide nucleic acid (PNA)-oligomer, said oligomer consisting essentially of essentially of at least one base sequence having a length of at least 10 nucleotides which hybridises to or is identical to one of the nucleic acid sequences according to Seq. ID No. 1 through Seq. ID No. 370.
5. The oligomer as recited in Claim 4, wherein the base sequence includes at least one CpG dinucleotide.
6. The oligomer as recited in Claim 5, characterised in that the cytosine of the CpG dinucleotide is located in the middle third of the oligomer.
7. A set of oligomers, comprising at least two oligomers according to any of claims 4 to 6.
8. A set of oligomers as recited in Claim 7, comprising oligomers for detecting the methylation state of all CpG dinucleotides within the group of Seq. ID No.1 through Seq. ID No. 73 and Seq.

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ID No. 366 and sequences complementary thereto.

9. A set of at least two oligonucleotides as recited in one of Claims 4 through 8, which is used as primer oligonucleotides for the amplification of nucleic acid sequences of one of Seq. ID No. 1 through Seq. ID No. 370 and sequences complementary thereto.
10. A set of oligonucleotides as recited in one of Claims 7 through 9, characterised in that at least one oligonucleotide is bound to a solid phase.
11. Use of a set of oligonucleotides comprising at least three of the oligomers according to any of claims 4 through 10 for detecting the cytosine methylation state and/or single nucleotide polymorphisms (SNPs) within the sequences taken from the group of Seq. ID No. 1 to Seq. ID No. 370 and sequences complementary thereto.
12. A method for manufacturing an arrangement of different oligomers (array) fixed to a carrier material for analysing breast cell proliferative disorders associated with the methylation state of any of the CpG dinucleotides of the group Seq. ID No.1 through Seq. ID No. 73 and Seq. ID No. 366, wherein at least one oligomer according to any of the claims 3 through 10 is coupled to a solid phase.
13. An arrangement of different oligomers (array) obtainable according to claim 12.
14. An array of different oligonucleotide- and/or PNA-oligomer sequences as recited in Claim 13, characterised in that these are arranged on a plane solid phase in the form of a rectangular or hexagonal lattice.
15. The array as recited in any of the Claims 13 or 14, characterised in that the solid phase surface is composed of silicon, glass, polystyrene, aluminium, steel, iron, copper, nickel, silver, or gold.
16. A DNA- and/or PNA-array for analysing breast cell proliferative disorders associated with the methylation state of any of the CpG dinucleotides of the group Seq. ID No.1 through Seq. ID No. 73 and Seq. ID No.366 comprising at least one nucleic acid according to one of the preceding claims.

17. A method according to Claim 1 or 2 characterised in that the following steps are carried out:
- a) in a genomic DNA sample, cytosine bases which are unmethylated at the 5-position are converted, by chemical treatment, to uracil or another base which is dissimilar to cytosine in terms of hybridisation behaviour;
 - b) amplifying at least one fragment of the pretreated genomic DNA using sets of primer oligonucleotides and a polymerase, wherein said fragments comprise one or more sequences taken from the group of Seq. ID No. 74 to Seq. ID No. 365 and SEQ ID NO: 367 to SEQ ID NO: 370 and sequences complementary thereto;
 - c) determining the methylation status of the genomic CpG dinucleotides by analysis of the amplificate nucleic acids.
18. The method as recited in Claim 17, characterised in that Step c) is carried out by means of hybridisation of at least one oligonucleotide according to Claims 3 through 10.
19. The method as recited in Claim 17, characterised in that Step c) is carried out by means of hybridisation of at least one oligonucleotide according to Claims 3 through 10 and extension of said hybridised oligonucleotide(s) by means of at least one nucleotide base.
20. The method as recited in Claim 17, characterised in that Step c) is carried out by means of sequencing.
21. The method as recited in Claim 17, characterised in that Step b) is carried out using methylation specific primers.
22. The method as recited in Claim 17, characterised in that Step c) is carried out by means of a combination of at least two of the methods described in Claims 18 through 21.
23. The method as recited in Claim 17, characterised in that the chemical treatment is carried out by means of a solution of a bisulfite, hydrogen sulfite or disulfite.
24. A method according to Claims 1 and 2 characterised in that the following steps are carried out:
- a) obtaining a biological sample containing genomic DNA,
 - b) extracting the genomic DNA,
 - c) digesting the genomic DNA comprising one or more of the sequences from the group

comprising Seq. ID No. 1 through Seq. ID No. 73 and Seq. ID No. 366 with one or more methylation sensitive restriction enzymes, and

d) detection of the DNA fragments generated in the digest of step c).

25. A method according to Claim 24, wherein the DNA digest is amplified prior to Step d).
26. The method as recited in one of the Claims 17 through 23 and 25 characterised in that more than ten different fragments having a length of 100 - 200 base pairs are amplified.
27. The method as recited in one of Claims 17 through 23, 25 and 26 characterised in that the amplification of several DNA segments is carried out in one reaction vessel.
28. The method as recited in one of the Claims 17 through 23 and 25 through 27, characterised in that the polymerase is a heat-resistant DNA polymerase.
29. The method as recited in one of the Claims 17 through 23 and 25 through 28, characterised in that the amplification is carried out by means of the polymerase chain reaction (PCR).
30. The method as recited in one of the Claims 17 through 23 and 25 through 29, characterised in that the amplicates carry detectable labels.
31. The method according to Claim 30 wherein said labels are fluorescence labels, radionuclides and/or detachable molecule fragments having a typical mass which can be detected in a mass spectrometer.
32. The method as recited in one of the Claims 17 through 23, characterised in that the amplicates or fragments of the amplicates are detected in the mass spectrometer.
33. The method as recited in one of the Claims 31 and/or 32, characterised in that the produced fragments have a single positive or negative net charge for better detectability in the mass spectrometer.
34. The method as recited in one of Claims 30 through 33, characterised in that detection is carried out and visualised by means of matrix assisted laser desorption/ionisation mass spectrometry

(MALDI) or using electron spray mass spectrometry (ESI).³⁰

35. The method as recited in one of the Claims 17 through 30, characterised in that the genomic DNA is obtained from cells or cellular components which contain DNA, sources of DNA comprising, for example, cell lines, histological slides, biopsies, tissue embedded in paraffin and all possible combinations thereof.
36. A kit comprising a bisulfite (= disulfite, hydrogen sulfite) reagent as well as oligonucleotides and/or PNA-oligomers according to one of the Claims 4 through 10.
37. A kit according to claim 36, further comprising standard reagents for performing a methylation assay from the group consisting of MS-SNuPE, MSP, Methyl light, Heavy Methyl, nucleic acid sequencing and combinations thereof.
38. The use of a method according to one of claims 1, 2, 12, and 17 to 35, a nucleic acid according to Claim 3, of an oligonucleotide or PNA-oligomer according to one of the Claims 4 through 10, of a kit according to Claim 36 or 37, of an array according to one of the Claims 14 through 16 or of a set of oligonucleotides according to one of claims 7 through 10 for the characterisation, classification, differentiation, grading, staging, and/or diagnosis of breast cell proliferative disorders, or the predisposition to cell proliferative disorders.
39. The use of a method according to one of claims 1, 2, 12, and 17 to 35, a nucleic acid according to Claim 3, of an oligonucleotide or PNA-oligomer according to one of the Claims 4 through 10, of a kit according to Claim 36 or 37, of an array according to one of the Claims 14 through 16 or of a set of oligonucleotides according to one of claims 7 through 10 for the therapy of breast cell proliferative disorders.